# BIODEGRADABLE BIOCOMPOSITE STARCH BASED FILMS BLENDED WITH CHITOSAN AND WHEY PROTEIN

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## ABSTRACT

The use of synthetic plastic materials as a food packaging causes serious hazards to the environment. The introduction of biodegradable materials, which can be disposed directly into the soil, can be one possible solution to this problem. The objectives of this work were to produce biodegradable biocomposite films and study the characterization of starch-based films blended with chitosan and whey protein. The films were synthesized by using the mixing process and the casting method. The characteristics of the blend films with different tapioca starch composition (1, 2, 3, 4 and 5 g/100 mL) were evaluated using Universal Testing Machine, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimeter (DSC), Thermogravimetric Analysis (TGA), scanning electron microscope (SEM) observation and biodegradability using microbiological degradation test and soil burial degradation test. Among them, the biodegradable blend films compatibilized with 1% of tapioca starch content showed good mechanical properties and had the highest thermal stability. The FTIR confirmed that tapioca starch, chitosan and WPI were compatible and inter-molecular hydrogen bonds existed between them. Moreover, the SEM analysis with 1% of tapioca starch content showed a compatible, smooth and homogenous structure of the composite film. The microbiological degradation test indicated that the growth of *A. Niger* colony increases as the tapioca starch content was increased. In soil burial test, a rapid degradation occurred for all the films in the initial 10 days followed by 100% composting within 18 days. As a conclusion, the film fabricated had potential application in future to be used as food packaging because it can enhanced foods quality and at the same time protected the environment.

# ABSTRAK

Penggunaan bahan sintetik plastik sebagai pembungkus makanan boleh menyebabkan masalah yang serius terhadap persekitaran. Pengenalan bahan bio-urai dimana ia boleh dibuang secara terus ke tanah, boleh menjadi salah satu penyelesaian untuk masalah ini. Tujuan kajian ini dijalankan adalah untuk menghasilkan filem biokomposit biodegrdadasi dan mengkaji ciri-ciri filem berasaskan kanji dicampur dengan kitosan dan tepung protein dadih (WPI). Filem ini dihasilkan dengan menggunakan proses pencampuran dan kaedah tuangan. Filem yang terhasil dengan komposisi campuran kanji ubi kayu yang berbeza (1, 2, 3, 4 dan 5 g/100 mL) ini telah dicirikan dengan menggunakan mesin pengujian, spektroskopi inframerah transformasi Fourier (FTIR), kalorimeter pengimbasan perbezaan (DSC), analisi termogravimetri (TGA), mikroskopi pengimbasan elektron (SEM) dan biodegradasi menggunakan kaedah mikrobiologi dan kaedah timbus tanah. Di antara filem yang telah dihasilkan, kanji ubi kayu dengan komposis 1% menunjukkan sifat mekanik yang kukuh dan mempunyai kestabilan terma yang tertinggi. FTIR menegaskan bahawa kanji ubi kayu, kitosan dan WPI bersesuaian dan wujud ikatan hidrogen diantaranya. Tambahan pula, SEM analisis bagi komposisi 1% kanji ubi kayu menunjukan struktur yang serasi, halus dan homogen. Uji kaji degradasi mikrobiologi menunjukkan bahawa pertumbuhan koloni *A. Niger* meningkat dengan pertambahan komposisi kanji ubi kayu. Dalam uji kaji timbus tanah, degradasi pantas berlaku untuk semua filem pada 10 hari terawal diikuti dengan 100% kompos dalam masa 18 hari. Kesimpulannya, filem yang terhasil ini mempunyai aplikasi berpotensi di masa hadapan untuk digunakan sebagai pembungkus makanan kerana ia dapat meningkatkan kualiti makanan dan dalam masa yang sama dapat melindungi alam sekitar.

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| | | |
|---|---|---|
| $^{\circ}C$ | - | Celsius |
| *CD* | - | Compact disk |
| *DSC* | - | Differential Scanning Calorimetry |
| *E* | - | Elongation or modulus of elasticity |
| *FTIR* | - | Fourier Transformed Infrared Spectroscopy |
| $\Delta H$ | - | Melting or gelatinization enthalpy |
| *SEM* | - | Scanning Electron Microscopy |
| *Tc* | - | Crystallization point |
| $T_d$ | - | Decomposition temperature |
| *Tg* | - | Glass transition temperature |
| *TGA* | - | Thermogravimetric Analysis |
| *Tm* | - | Melting temperature |
| *TS* | - | Tensile Strength |
| *TV* | - | Television |
| *WPI* | - | Whey protein isolates |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Study

Nowadays, million tons of plastic are produced annually all over the world. The production and consumption continue to increase each year. It plays a significant role in our daily life and has become a widely used material due to its wide array of applications particularly as packaging materials.

Petrochemical based plastics such as polyolefin, polyesters and polymides have been increasingly used as packaging materials because of their availability in large quantities at low cost and favorable functionality characteristics such as good tensile and tear strength, good barrier properties to oxygen and aroma compounds and heat seal ability. However, these plastics are made of petroleum-based materials that are not readily biodegradable and therefore lead to environmental pollution, which pose serious ecological problem (Ali *et al*., 2008). The most obvious form of pollution associated with plastic packaging is waste plastic dump to the landfills. Plastics are very stable and therefore settle in the environment in prolong time after been discarded, especially if they are shielded from direct sunlight by being buried in landfills. It can leach harmful chemicals that eventually spread into groundwater sources which may cause numerous illnesses to human health.

In order to overcome these problems, several studies are concentrated on the development of new biodegradable packing materials that can be manufactured with the utilization of environmentally friendly raw materials. Among the natural

polymers, starch has been considered as one of the most promising candidates for future materials because of its attractive combination of price, abundance and renewable in addition to biodegradability. Starch naturally occurs in a variety of botanical sources such as wheat, corn, yam, potatoes and tapioca (Salleh and Muhamad, 2008). The preponderance of amylose in starch increases the strength of the films. However, biodegradable products based on starch, possess many disadvantages, mainly attributed to the water solubility, brittle nature of starch films and poor mechanical properties. Therefore, it needs to be treated first by either plasticization or blending with other materials.

Plasticizers are added to polymers to reduce brittleness, since they work as spacers polymer chains, decreasing inter-molecular forces and thus increasing flexibility and extensibility of polymers. They must be compatible with the film forming polymers (Wu *et al*., 2009). Common plasticizers for starch are glycerol and other low-molecular-weight-polyhydroxy-compounds, polyethers and urea. Glycerol was the most suitable plasticizer with respect to mechanical properties and transparency.

Moreover, one of the effective strategies to overcome the poor mechanical properties of starch based films, while preserving the biodegradability of the material, is to associate starch with chitosan. Chitosan, a 1,4 linked-2-deoxy-2-aminoglucose, prepared by *N*-deacetylation of chitin (Xu *et al*., 2005). It is relatively low cost, widespread availability from a stable renewable source, that is, shellfish waste of the sea food industry (Bourtooma and Chinnan, 2007) and appears as a natural antimicrobial candidate for the incorporation because it can inhibit the growth of a wide variety of fungi, yeasts and bacteria (Salleh and Muhamad, 2008).

Whey protein is the collection of globular proteins isolated from whey, a by-product of cheese manufactured from cow's milk. It can be used for production of biodegradable films. Whey protein films have low tensile strength and high water vapor permeability due to the high proportion of hydrophilic amino acid in their structures (Ghanbarzadeh and Oromiehi, 2008). According to B. Sen Gupta and Magee (2007), these films were found to retard moisture loss and oxygen diffusion.

It also showed good tensile strength and moderate elongation. The films had apparently no flavor or taste that interfered with those of the food. The films also have excellent mechanical and barrier properties.

Hence, this study will focus on the preparation and characterization of biodegradable blend films from starch-chitosan-whey protein as well as their microstructure, thermal properties and biodegradibility. The method applied to produce starch-chitosan-whey protein blend films is by using casting technique.

## 1.2 Problem Statement

Plastic is the "highlander" material, living extremely long lives, used in countless products that could potentially have service over decades. The production and disposal of plastics contribute to an array of environmental problems, which pose serious ecological problem. The most obvious form of pollution associated with plastic packaging is waste plastic sent to landfills. Plastics are very stable and therefore settle in the environment in prolong time after been discarded, especially if they are shielded from direct sunlight by being buried in landfills. It can leach harmful chemicals that eventually spread into groundwater sources which may cause numerous illnesses to human health. Other than that, we are also facing problem with the depletion of fossil fuels sources, which are connected to plastics as their raw materials. Crude oil is a product of fossils and therefore it is not renewable. Hence, a possible solution to overcome the problems is by using biodegradable composite. Thus by blending starch, chitosan and whey protein it would produce bio-composite film which offer biodegradability and subsequently reduce the amount of pollution caused by polymers to the environment.

## 1.3 Research Objective

The objectives of this research are to produce biodegradable biocomposite films and to study the characteristics of starch-based films blended with chitosan and whey protein.

## 1.4 Scopes Of The Study

The scopes of this study are:

1. To produce biodegradable biocomposite films from tapioca starch blended with chitosan and whey protein.
2. To investigate the influence of starch composition on biodegradable biocomposite films (1, 2, 3, 4 and 5 g/ 100 mL).
3. To determine the mechanical properties of the biodegradable blend films using Universal Testing Machine.
4. To identify the interactions and/or chemical bonds between tapioca starch, chitosan and whey protein by using Fourier Transform Infrared Spectroscopy (FTIR).
5. To study the thermal properties and stability of blend films by using Thermogravimetric Analysis (TGA) and Differential Calorimetry Scanning (DSC).
6. To observe the microstructure of films by using scanning electron microscope (SEM).
7. To determine the biodegradability of blend films by microbial degradation using *Aspergillus Niger* and soil burial degradation test.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Polymers: Synthetic Polymers and Natural Polymers

Polymers are long chain giant organic molecules that are assembled from many smaller molecules called monomers. Polymers consist of many repeating monomer units in long chains. A polymer is analogous to a necklace made from many small beads (monomers). Another common name for many synthetic polymers is plastic which comes from the Greek word "plastikos", suitable for molding or shaping. Many objects in daily use from packing, wrapping and building materials include half of all polymers synthesized. Other uses include textiles, TV's, CD's, automobiles and many other all are made from polymers. There are many types of polymers including synthetic and natural polymers (Ophardt and Losey, 2003).

Synthetic polymers can be classified as addition polymers, formed from monomer units directly joined together or condensation polymers, formed from monomer units combining such that a small molecule, usually water, is produced during each reaction (Joesten, 2008). Some examples of synthetic polymers are polyethylene, polyester, polyurethane and many more. These types of polymers basically cannot be degraded and thus need to be recycle in order to avoid landfills problems.

Natural polymers are large molecules, produced by plants and animals that carry out many life-sustaining processes in a living cell. The cell membranes of plants and the woody structure of trees are composed in large part of cellulose, a

polymeric carbohydrate. Carbohydrates, which comprise one of the three classes of foodstuffs, contain carbon, hydrogen and oxygen atoms. They can be classified as monosaccaharides, disaccharides and polysaccharides (Moore *et al*., 2005).

## 2.2 Film Forming Materials

Film forming materials, such as starches, chitosan, proteins and glycerol are used for different purposes in a biomaterial and/or edible film preparation process. Biopolymers like starches and proteins create the basic network structure of the film. However, films prepared from biopolymers are often too fragile to stand handling, *e.g.*, bending or stretching. Thus, they have to be plasticized using low molecular weight substances, such as polyols (glycerol, xylitol, sorbitol, sugar and maltitol), which decrease interactions between the biopolymer chains (Wu *et al*., 2009).

Biopolymers used in film preparation are often carbohydrates or proteins extracted or separated from plants, animal tissues or animal products. The storage carbohydrate in plants is starch. Depending on the plant the starch is formed in different parts of the plant, *e.g.*, grain, tuber or root. Other carbohydrates found in the plants *e.g.* the cell wall include cellulose and pectin (Keeling, 1998). Some carbohydrates, such as alginate and carrageenan, are found in seaweeds (Larotonda *et al*., 2005). Biopolymers extracted from animal products or parts are also used in edible film manufacturing. Chitosan is widespread available in shellfish waste of the sea food industry and have an ability to form a good films (Bourtooma and Chinnan, 2007). Casein and whey proteins are separated from milk and they are often used to prepare films (Ghanbarzadeh and Oromiehi, 2008) and (B. Sen Gupta and Magee, 2007).

Out of all of the film forming materials mentioned in this section, this literature review focuses mainly on the starch, chitosan, whey protein and polyols.

### 2.2.1 Starch

#### 2.2.1.1 Sources for Starch

Starch is the major carbohydrate reserve in plant tubers and seed endosperm where it is found as granules (Buleon *et al*., 1998), each typically containing several million amylopectin molecules accompanied by a much larger number of smaller amylose molecules. By far the largest source of starch is corn (maize) with other commonly used sources being wheat, potato, tapioca and rice. Amylopectin (without amylose) can be isolated from 'waxy' maize starch whereas amylose (without amylopectin) is best isolated after specifically hydrolyzing the amylopectin with pullulanase (Vorwerg *et al*., 2002). Genetic modification of starch crops has recently led to the development of starches with improved and targeted functionality (Jobling, 2004).

#### 2.2.1.2 Starch Structure and Composition

Starch molecules arrange themselves in the plant in semi-crystalline granules. Each plant species has a unique starch granular size: rice starch is relatively small, about 2μm, and potato starch has larger granules about up to 100μm (Wikipedia). Native starches from different botanical sources vary widely in structure and composition, but all granules consist of two major molecular components, amylose (20-30%) and amylopectin (70-80%), both of which are polymers of α-D-glucose units in the $C_1$conformation. In amylose (Refer Figure 2.1), these are linked -(1 → 4)-, with the ring oxygen atoms all on the same side, whereas in amylopectin about one residue in every twenty is also linked -(1 → 6)- forming branch-points as shown in Figure 2.2 (Nowjee, 2004).

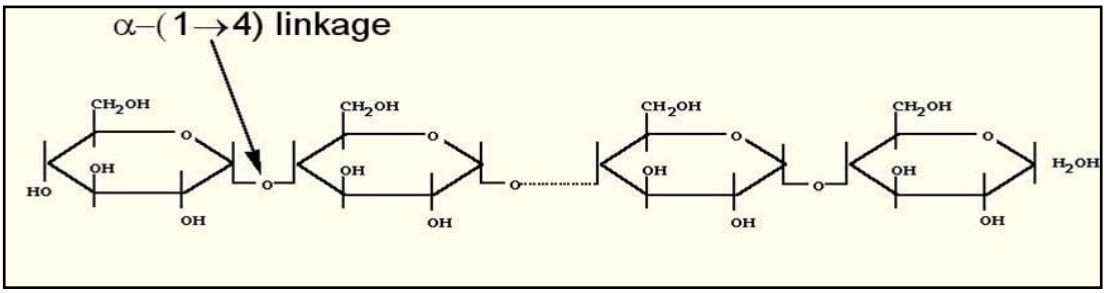


**Figure 2.1:** Amylose molecules (Nowjee, 2004)

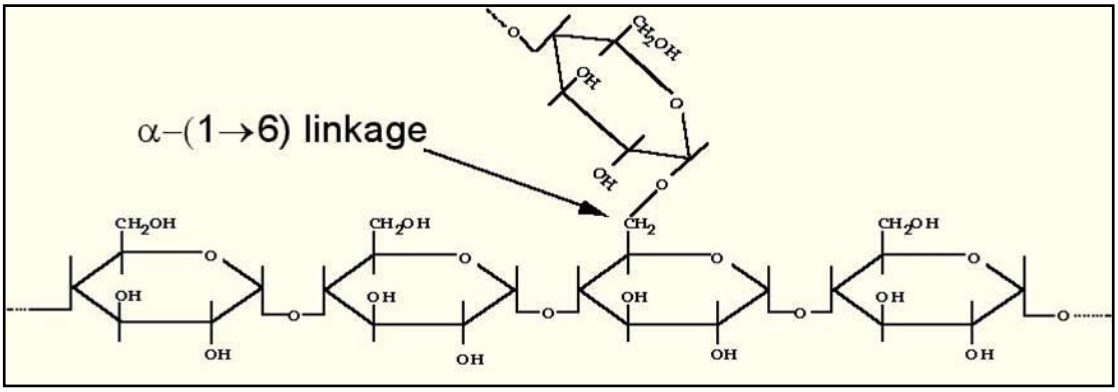


**Figure 2.2:** Amylopectin molecules (Nowjee, 2004)

A typical feature of starch is that it becomes soluble in water when heated. The granules swell and burst, the semi-crystalline structure is lost and the smaller amylose molecules start leaching out of the granule. This process is called starch gelatinization. During cooking the starch becomes a paste and gets its viscosity. During cooling or prolonged storage of the paste, the semi-crystalline structure partially recovers and the starch paste thickens. This is mainly caused by the retrogradation of the amylose. This process is also responsible for the staling, hardening of bread and the water layer on top of a starch gel (syneresis). Some cultivated plant varieties have pure amylopectin starch without amylose, known as waxy starches. The most used is waxy maize. Waxy starches have less retrogradation, the viscosity of the paste will be more stable. Also high amylose starch, amylomaize, is cultivated for the use of its gel strength (Wikipedia).

### 2.2.2 Chitin and Chitosan

#### 2.2.2.1 Structural and Characterization of Chitin and Chitosan

Chitin and chitosan are known biodegradable natural polymers based on polysaccarides, which are extracted from various animals and plants. Chitin exists widely in cell walls of some microorganisms such as fungi, molds and yeasts and in the cuticular and exoskeletons of invertebrates such as crustaceans, mollusks, crab, shrimps, lobster, squid and insects (Knaul *et al.*, 1999). Chitosan exists naturally only in a few species of fungi. Chitin and chitosan consist of 2-acetamido-2-deoxy-β-D-glucose and 2-amino-2-deoxy-β-Dglucose as repeating units respectively. Chitin is chemically identical to cellulose except that secondary hydroxyl group on the alpha carbon atom of the cellulose molecule is substituted with acetoamide groups (Refer Figure 2.3).

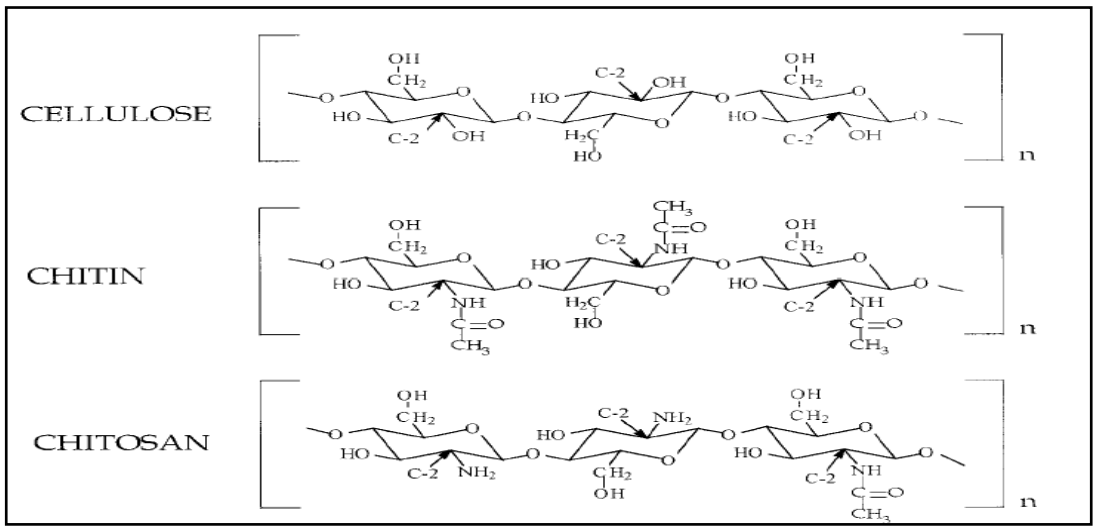


**Figure 2.3:** Comparison of Molecular Structure of Cellulose, Chitin, Chitosan (Knaul *et al*., 1998).

Chitosan is the N-acetylated form of chitin. Figure 2.4 shows the deacetylation reaction of chitin.

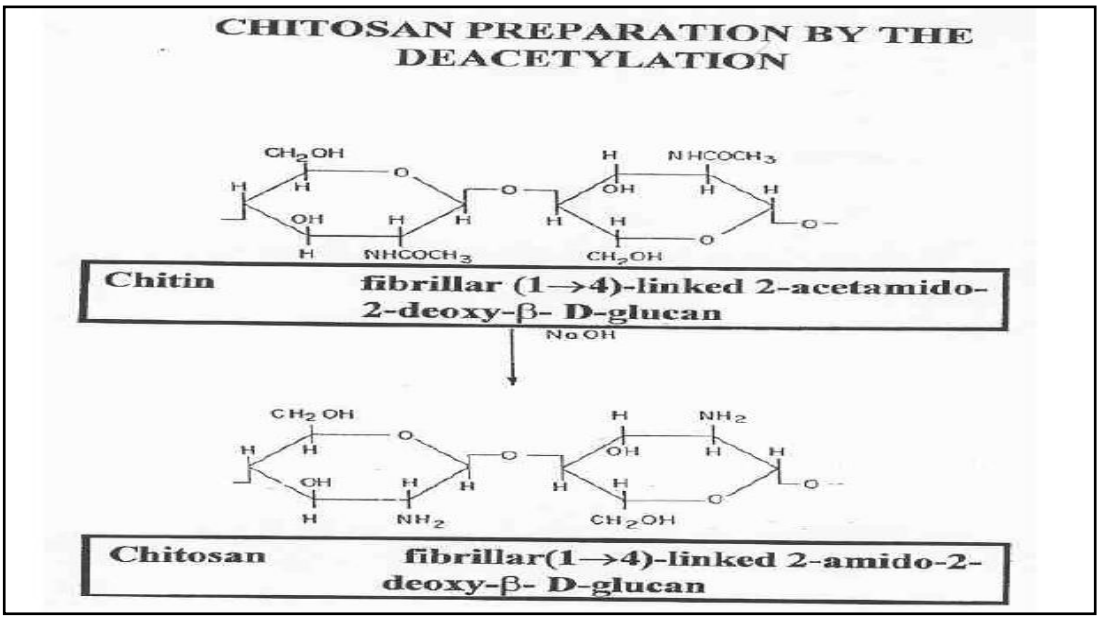


**Figure 2.4:** The chitosan production with deacetylation of chitin (Melichar, 2007).

Chitosan is insoluble in water and is soluble in acidic solvent below pH 6. Organic acid such as acetic formic and lactic acids are used for dissolving chitosan and most commonly used is 1% acetic acid solution (pH is about 4.0). Solubility in inorganic acids is quite limited. Chitosan is soluble in 1 % hydrocloric acid but insoluble in sulfuric and phosphoric acids. Chitosan solution's stability is poor above about pH 7. At higher pH, precipitation or gelation will occur. Chitosan solution forms poly-ion complex with anionic hydrocolloid and provides gel. Figure 2.5 shows the chitosan preparation from chitin and chitosan solution in acetic solvent (Onar and Sariisik, 2002).

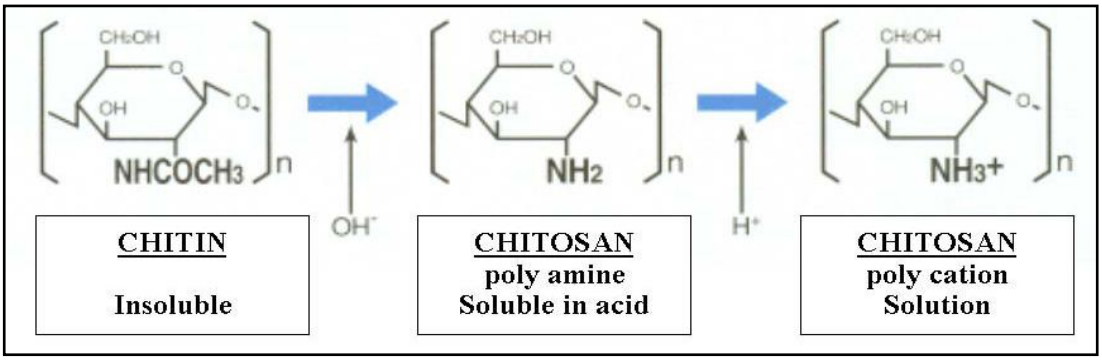


**Figure 2.5:** Chitosan preparation from chitin and chitosan solution in acidic solvent (Onar and Sariisik, 2002).

### 2.2.2.2 Chitin and Chitosan Manufacturing Process

In most studies, chitin and chitosan fibers are produced by a wet-spinning process, but rarely by a dry-spinning process. When using wet-spinning process to produce the fibers, the two polymers firstly are dissolved in a solvent and then the polymer solution is extruded via fine holes (especially through a viscose-type spinneret) into a non-solvent (coagulant) at 45-50 0C. The polymer precipitates out in the form of a filament, which can be washed, drawn and dried to form the fibers (Onar and Sariisik, 2002). Figure 2.6 shows the chitin and chitosan manufacturing process.

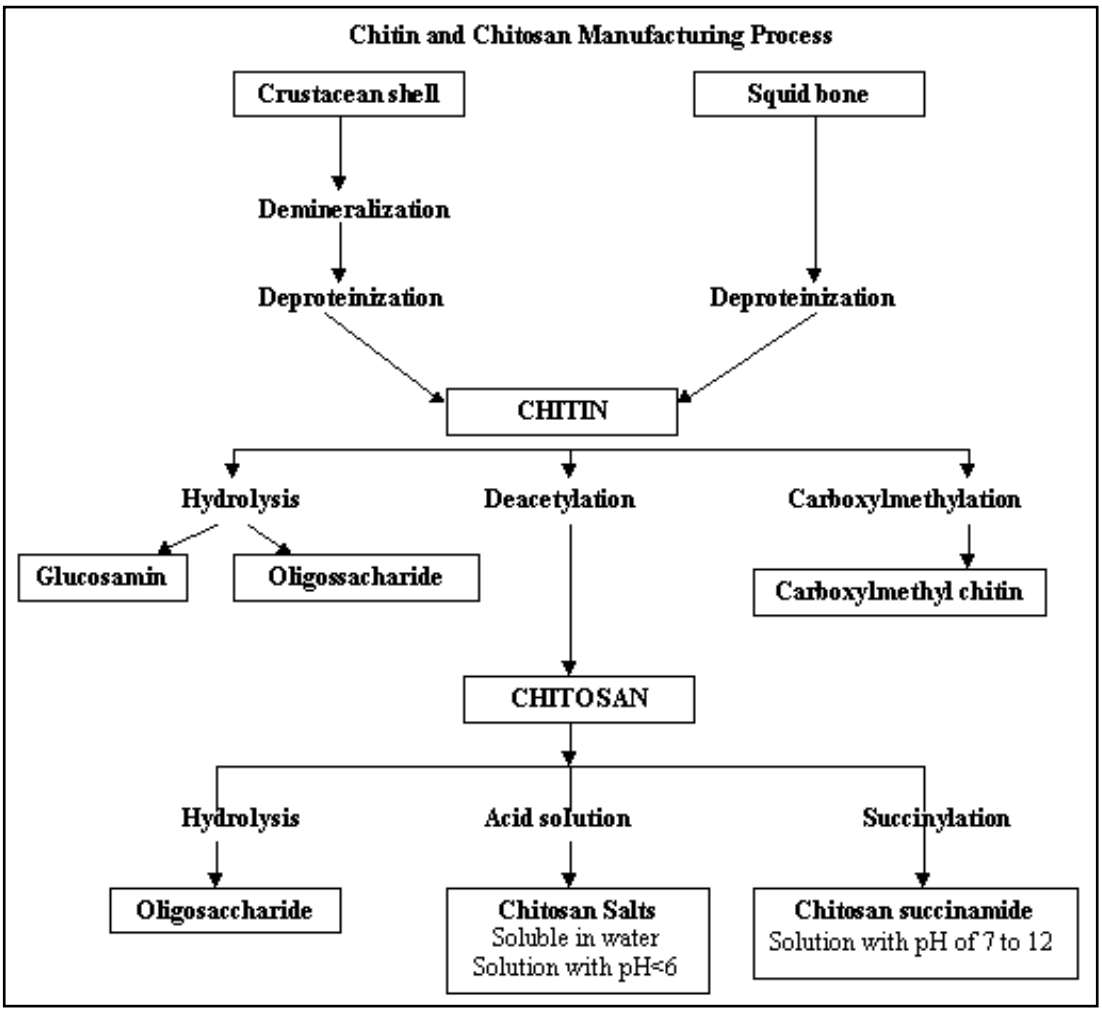


**Figure 2.6:** Chitin and chitosan manufacturing process (Onar and Sariisik, 2002).

### 2.2.3 Whey Protein

Whey is a by-product of the cheese making process. Production of a kilogram of cheese yields about 9 kilograms of liquid whey. The exact composition of whey is dependent on the source of milk and the manufacturing process. It commonly consists of a rich blend of lactose, proteins and minerals and can be used as a low cost source of proteins, carbohydrates vitamins and minerals. Normal milk contains about 78% of caseins, the remainder being the whey proteins as shown in Table 2.1. Raw whey is not suitable for direct consumption owing to high levels of water (93.5%) and lactose (4.5-5.0%). Usually whey is dried to a powder form for food related applications, in particular as a protein supplement in processed food. Whey proteins can be concentrated by ultrafiltration. The product is used extensively in processed food and beverages as a protein supplement. In addition to their nutritional values, whey proteins improve rheological properties of the food material during processing (B. Sen Gupta and Magee, 2007).

**Table 2.1:** Typical composition of Whey Protein
(B. Sen Gupta and Magee, 2007)

| Protein | Concentration, kg/m$^3$ | pH |
|---|---|---|
| β- Lactoglobin | 2.7 | 5.2 |
| α- Lactalbumin | 1.2 | 4.5 |
| Immunoglobins | 0.65 | 5.5 |
| Bovine serum | 0.4 | 4.7 |
| Lactoferrin | 0.1 | 9.0 |

Whey proteins produce transparent, bland, flexible water-based edible films with excellent oxygen, aroma and lipid barrier properties at low relative humidity (Perez-Gago and Krochta, 2000). They also show good tensile strength and moderate elongation (B. Sen Gupta and Magee, 2007). Whey protein- based films have an extra edge over many other biopolymer films because of excellent nutritional value, bland flavor and capability to carry flavorings and functional ingredients (Li and Chen, 2000).

### 2.2.4 Polyols

Polyols (polyalcohols) are low molecular weight carbohydrates which are used in food, non-food, health care and pharmaceutical applications. They are increasingly used to provide the sweetness of various products or replace sucrose in confectionery. Polyols are used in chewing gum, because they do not contribute to development of dental caries and they neutralize pH in the mouth. Moreover, polyols are also used as plasticizers in the edible films.

Commonly, polyols are produced by the hydrogenation process in which hydrogen is added to the carbonyl group of saccharides. Depending on the starting materials of the hydrogenation process polyols are divided into three categories which are hydrogenated monosaccharides, hydrogenated disaccharides and mixtures of hydrogenated polysaccharides. The hydrogenation process of monosaccharides, such as D-glycerose, D-xylose, D-glucose and D-mannose, yields glycerol, xylitol, D-glucitol (sorbitol) and mannitol, respectively and correspondingly the hydrogenation process of disaccharides, such as maltose or lactose, yields maltitol and lactitol, respectively. Most of the polyols, such as erythritol, xylitol and sorbitol, appear as crystalline powders which have their characteristic melting temperatures, whereas glycerol is a melt (Talja, 2007).

Monosaccharide-based polyols, such as glycerol and sorbitol, are widely used as plasticizers in edible film applications because of their plasticization ability due to their low molecular weights. Plasticizer is added to the film to give better handling properties like flexibility and elasticity. Plasticizer decreases interactions between biopolymer chains, such as amylose and amylopectin, thus preventing their close packing which results in lower degree of crystallinity in the film. Pores and cracks in the film could be also prevented by using plasticizers. Polyols are good plasticizers because of their low molecular weight and *Tg*. Generally, the lower the *Tg* of the plasticizer the less it will be needed to obtain plasticized film. This is fairly important because at the high plasticizer content phase separation of the plasticizer may occur (Talja, 2007). Table 2.2 shows the characteristic properties of various monosaccharide- and disaccharide-based polyols.